expression that supports dorsal–ventral patterning within the neural tube as well as proliferation and differentiation. Cephalic neural tube development differs in that gene expression supports synchronistically the closing neural tube with optic invagination and segmental organization of the brain. BMPs have been shown to play a role in neural tube development particularly as dorsalizing factors with well-established expression patterns in the surface ectoderm near the dorsal portion of the neural tube. Analysis of BMP2 chimeras generated from BMP2 null ES cells injected to wild type blastocysts revealed that embryos display both open and closed neural tube defects in the cephalic region. Exclusion of BMP2 null ES cells from the dorsal portion of the neural tube did not always prevent defects. Together with the morphological and molecular analysis of heterozygous and homozygous null BMP2 embryos, our results indicate that BMP2 is required for normal cephalic neural tube closure and future brain development.

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BMP and nodal signaling act synergistically in mammalian rostral patterning

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Holoprosencephaly (HPE) is a common and often lethal syndrome of forebrain and craniofacial midline defects. The molecular and cellular causes of HPE are poorly understood; however, mutations in the Shh and Nodal signaling pathways have been associated with HPE both in human and mice. In mouse, genetic reduction of antagonists of Bone Morphogenetic Protein (BMP) signaling also results in HPE. Based on the inheritance patterns of human HPE, we hypothesize HPE often results from compromises of independent loci or developmental pathways. We use mouse mutations to explore the effect of elevated BMP signaling with compromised Nodal signaling on early head development. We found that embryos with mutations in *Nodal* and the BMP antagonist *Chordin* exhibit HPE at moderate penetrance. To test whether this synergistic phenotype was a result of reduced Nodal signaling and BMP antagonism, we prepared an analogous double mutants bearing mutations affecting the same pathways: mutants for a downstream mediator of Nodal signaling, *Smad3*, and a second BMP antagonist, *Noggin*. *Nog;Smad3* mutants showed severe HPE at about 50% penetrance. Affected *Chrd;Nodal* and *Nog;Smad3* embryos show a loss of *Shh* and *Goosecoid* expression in midline of ventral forebrain, suggesting defects in the prechordal plate. Defects in rostroventral foregut was revealed by loss of *Hex* expression. These data indicate that Nodal and BMP antagonism act synergistically in patterning the ventral head and foregut, supported by additional molecular data. They also suggest that human birth defects of the forebrain and facial midline can result from patterning errors that occur during early gastrulation.

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Characterization of a novel gene expressed exclusively in the zone of polarizing activity in the vertebrate limb

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Classical experiments identified the zone of polarizing activity (ZPA) as being responsible for patterning the anteroposterior limb axis. Subsequent experiments determined that Shh is responsible for the polarizing activity associated with this region of the developing limb. To date, Shh is the only gene known to be expressed exclusively in the ZPA in the limb. To identify other genes expressed in the ZPA, we took advantage of a transgenic mouse allele in which we had inserted GFP into the Shh locus. Embryos with this transgene expressed GFP in all cells that normally express Shh. Cells expressing Shh were cell sorted from E10.5 limbs and labeled complementary RNAs from GFP-positive (the ZPA) and GFP-negative cell populations were hybridized to Affymetrix GeneChips. Analysis of the data revealed that Shh and 15 other genes were expressed at higher levels in the ZPA. One of these genes, an uncharacterized EST with 8 transmembrane domains, was analyzed further. We have provisionally named this gene TM1. Whole mount RNA in situ hybridization revealed that TM1 is expressed in the ZPA prior to Shh. It then is co-expressed with Shh during limb development. No other expression is found in the limb. TM1 is a member of a family of transmembrane proteins that share no similarity to any of the known transmembrane proteins functioning in the Shh-signaling pathway. RNA in situ hybridization analysis of TM1 expression in mutant mouse embryos suggests that Fgf-signaling is necessary for normal TM1 expression, but Shh is not. We have recently constructed a mouse containing a TM1 null allele. The analysis of TM1 loss-of-function mice will be presented.

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Ndst1 is required for FGF signaling in early lens development

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Previous studies have implicated multiple signaling molecules, including bone morphogenic proteins (BMP) and fibroblast growth factors (FGF), in early lens development. However, regulation of these morphogen is still largely unknown. As essential components of major signaling pathways, heparan sulfate is known to participate in both morphogen transport and morphogen–receptor interaction. In this study, we demonstrate that inactivation of the heparan sulfate biosynthetic gene Ndst1 results in early lens invagination defects, leading to severe lens hypoplasia or anophthalmia. Although Pax6 and Six3 are not affected, AP2aα, Pitx3, Prox1 and αaA crystallin expressions are disrupted in the mutant lens. We show that the Ndst1 mutants are not defective in BMP or Wnt signaling. Instead, these embryos exhibit reduced sulfation level of heparan sulfate, and diminished binding to FGF ligand or FGF/FGF receptor complex. Consistent with disruption of FGF signaling, expressions of phospho-ERK and ERM expression are also downregulated in Ndst1 mutant lens. Taken together, these results establish the important role of Ndst1 function in FGF signaling during lens development.

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Hoxa1-lineage analysis suggests novel domains of Hoxa1 function

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Hox genes play important roles in regulating embryonic patterning and organogenesis. Hoxa1, one of the earliest and most anteriorly expressed Hox genes, is crucial for proper development of the brainstem, inner ear, cranial ganglia and cardiovascular system. To determine the contribution of Hoxa1-lineage to each of these structures, we performed genetic lineage analysis using the Cre/lox system. An IRES-Cre cassette was targeted to the 3′ untranslated region of Hoxa1, allowing Cre expression in the Hoxa1 domain while preserving endogenous gene function. Our results demonstrate that Hoxa1 expressing cells give rise to rhombomere 4-derived neural crest cells, which populate the second branchial arch and contribute to the VII/VIIIth ganglion complex. Hoxa1-lineage was also found in the otic epithelium and the heart. Additionally, we show that Hoxa1-lineage is present in the third rhombomere and does not exhibit a sharp anterior border at the rhombomere 3/4 boundary.

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Rbm19 is essential for nucleologensis in the early mouse embryo

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Rbm19 is a multiple RNA recognition motif (RRM)-containing protein that was identified in a forward genetic screen for digestive organ mutants in zebrafish (***Development*** 130: 3917), and independently isolated in a molecular screen for novel RRM proteins. It is required for pre-rRNA processing, but its exact biochemical function is unknown. To elucidate the role of Rbm19 in mammalian development, we generated Rbm19-mutant mice from a gene-trap insertion ES cell line. The insertion causes a truncation of the protein, deleting only the C-terminal (but most conserved RRM-containing domain). Embryos homozygous for the Rbm19 mutation arrest in their development at the morula stage. Electron microscopy revealed that E3.5 mutant blastomeres contain multiple electron dense spheres located in the periphery of the nucleus, in contrast to differentiated nucleoli seen in the heterzygote and wild-type embryos. Immunostaining for the nucleolar markers B23 and fibrillarin show altered expression patterns in the mutant. These results indicate that Rbm19 is a critical factor for early mouse development, and we attribute the early development arrest to failed nucleologenesis. Taken together with similar phenotypes in fibrillarin and pescadillo (PES-1) mouse mutants, these data reinforce the critical role of nucleologenesis in early mouse development.

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Characterization of zic1 and zic2 expression in early chick embryos

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Zic family members are transcription factors with multiple roles in early neural development. Zic genes are highly conserved, particularly in their zinc finger domains and in the regions immediately surrounding the zinc fingers. The chick genome suggests that zic4 and zic1 are adjacent and transcribed in opposite directions, as in mouse. zic2 and zic3 are on separate chromosomes, and there is no zic5 gene. We have generated in situ probes that are specific for the zic1 and zic2 genes in chick. While there is considerable overlap in the expression patterns of these two genes, there are also distinct differences. zic2 is strongly expressed in the entire dorsal neural tube, including the brain, trunk, and the neural folds that have not yet completely closed. zic1 expression is strong in the dorsal brain but very weak in the dorsal neural tube of the trunk. Thus, zic2 may be the principal zic gene expressed in the trunk dorsal neural tube. During somite development, zic1 expression in the dorsomedial somites begins at SS VIII–XI, while zic2 begins at SS VII–VIII. Both zic1 and zic2 are expressed strongly in the dorsomedial parts of more mature somites, particularly in the posterior portions. While zic1 is not expressed in the limb buds, zic2 is strongly expressed in nascent limb buds and continues to be expressed in the tips of the limb buds as their development progresses. We are currently analyzing the somite expression of zic1 and zic2 in more detail for any differences in their expression